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=> s l3 and human CD3
L4 55 L3 AND HUMAN CD3

=> s l4 and EpCAM
L5 9 L4 AND EPCAM

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L6 ANSWER 1 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN
2005:493633 Document No. 143:42686 Compositions comprising polypeptides specifically binding predetd. antigens and uses thereof. Hofmeister, Robert; Prang, Nadja; Wolf, Andreas; Hanakam, Frank; Urbig, Thomas; Itin, Christian; Baeuerle, Patrick (Micromet A.-G., Germany). PCT Int. Appl. WO 2005052004 A2 20050609, 49 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP13445 20041126. PRIORITY: EP 2003-27511 20031128.

AB The present invention relates to compns. comprising polypeptides capable of specifically binding predetd. antigens; the polypeptide in the composition comprises at least two antigen-binding sites. These at least two antigen binding sites are located on a single polypeptide chain. One of the at least two antigen binding sites specifically binds the **human CD3** antigen. The polypeptide may exist in both monomeric form and

multimeric form (usually dimeric). The multimeric form of the polypeptide constitutes no more than 5% of the total weight of the combined monomeric and multimeric forms of said polypeptide. One example presents activation of T cells by polypeptide in dimeric form in the absence of target cells. The results demonstrated that the dimeric polypeptide was able to activate T cells, whereas the monomer was not. These polypeptide compns. can be used for the prevention, treatment, or amelioration of diseases and disorders occurring in man.

L6 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN
2005:546858 Document No. 143:76820 **Bispecific single-chain antibodies** preparation and therapeutic uses thereof. Kufer, Peter; Berry, Meera; Baeuerle, Patrick; Itin, Christian (Germany). U.S. Pat. Appl. Publ. US 2005136050 A1 20050623, 12 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-743697 20031222.

AB The present invention discloses **bispecific antibodies** comprising two **antibody** variable domains on a single polypeptide chain, wherein a first portion of the **bispecific antibody** is capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen on the human immune effector cell, the first portion consisting of one **antibody** variable domain, and a second portion of the **bispecific antibody** specifically binding to a target antigen other than the effector antigen, the target antigen on a target cell other than the human immune effector cell, the second portion comprising one **antibody** variable domain (or 2 **antibody** variable domains). The inventors describe preparation and purification of a **bispecific antibody** with 3 **antibody** variable domains located on the same polypeptide chain. Progressing from the N to C terminus, the **antibody** contains: anti-human EpCAM VL; 15 amino acid linker of sequence (Gly4Ser)3; anti-human EpCAM VH; 5 amino acid spacer Gly4Ser; anti-**human CD3** VH; His6. This **antibody** showed activity as a recruiter of cytotoxic T cells as shown by the efficient killing of the target cells in a manner depending on the concentrate of the **bispecific antibody** added to a reaction mixture in the presence of cytotoxic T cells.

L6 ANSWER 3 OF 21 MEDLINE on STN
2005352035. PubMed ID: 16004801. Biologic properties of an anti-human ovarian carcinoma/anti-**human CD3 single chain bispecific antibody.** Yang Jie-Zuan; Zhang Zhong; Ma Li; Yao Xin-Sheng; Zhou Ming-Qian; Wang Xiang-Bin; Wang Xiao-Ning. (Institute of Molecular Immunology, Southern Medical University, Guangzhou, Guangdong, 510515, PR China.) Ai zheng = Aizheng = Chinese journal of cancer, (2005 Jul) Vol. 24, No. 7, pp. 787-91. Journal code: 9424852. ISSN: 1000-467X. Pub. country: China. Language: Chinese.

AB BACKGROUND & OBJECTIVE: Previous routine therapies can' t improve the survival rate of ovarian carcinoma patients. Experimental and pre-clinical data showed that **bispecific antibodies** could efficiently induce antitumor effect of cytotoxic cells. This study was to investigate the biologic properties of an anti-human ovarian carcinoma/anti-**human CD3 single chain bispecific antibody** (BHL-I) in vitro, and provide reference for pre-clinical experiment and its application. METHODS: Peripheral blood lymphocytes (PBLs), isolated from peripheral blood of healthy donors, were treated with BHL-I. The conjugation between PBLs and target ovarian carcinoma SKOV3 cells was observed under reverse microscope; the proliferation of peripheral blood mononuclear cells (PBMCs) and cytotoxicity of PBLs to SKOV3 cells were detected by MTT assay; the concentrations of human interferon-gamma (hIFN-gamma) and human tumor necrosis factor-alpha (hTNF-alpha), secreted by PBLs in the process of killing target cells, were detected by ELISA. RESULTS: The rosette (PBL-SKOV3) formation rate was significantly higher in BHL-I group than in control group (15.7% vs. 11.1%, P<0.01). BHL-I significantly enhanced the proliferation of PBLs and cytotoxicity of PBLs to SKOV3 cells in the

presence of relative antigen (P<0.01); the cytotoxic rate was positively correlated with the rosette formation rate (r=0.946); the concentrations of hIFN-gamma and hTNF-alpha were significantly increased (P<0.01). CONCLUSION: BHL-I could mediate conjugation between PBLs and SKOV3 cells, and activate the cytotoxicity of PBLs which may relate with up-regulation of hIFN-gamma and hTNF-alpha.

L6 ANSWER 4 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN
2004:1059395 Document No. 142:36927 **Bispecific single chain antibodies** against human EpCAM and **human CD3** antigen for cancer therapy. Kufer, Peter; Berry, Meera; Offner, Sonja; Brischwein, Klaus; Wolf, Andreas; Raum, Tobias; Kohleisen, Birgit; Lenkkeri-Schuetz, Ulla; Baeuerle, Patrick (Micromet A.-G., Germany). PCT Int. Appl. WO 2004106383 A1 20041209, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP5687 20040526. PRIORITY: EP 2003-12134 20030531; EP 2003-12133 20030531.

AB The present invention provides a pharmaceutical composition comprising a **bispecific single chain antibody** construct. Said **bispecific single chain antibody** construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to **human CD3** antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed **bispecific single chain antibody** construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

L6 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN
2004:1059393 Document No. 142:36925 Pharmaceutical compositions comprising **bispecific** anti-**human CD3**, anti-human CD19 **single chain antibodies** for treating B-cell related disorders. Kufer, Peter; Lutterbuese, Ralf; Kohleisen, Birgit; Zeman, Steven; Baeuerle, Patrick (Micromet A.-G., Germany). PCT Int. Appl. WO 2004106381 A1 20041209, 115 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP5685 20040526. PRIORITY: EP 2003-12136 20030531.

AB The present invention relates to a pharmaceutical composition comprising a **bispecific single chain antibody** construct, said **bispecific single chain antibody** construct comprising binding domains specific for **human CD3** and human CD19, wherein the corresponding variable heavy chain regions (VH) and the corresponding variable light chain regions (VL) regions are arranged, from N-terminus to C-terminus, in the order, VH(CD19)-VL(CD19)-VH(CD3)-VL(CD3), VH(CD3)-VL-(CD3)-VH(CD19)-VL(CD19) or VH(CD3)-VL(CD3)-VL(CD19)-VH(CD19). Furthermore, processes for the production of said pharmaceutical compns. as well as

medical/pharmaceutical uses for the specific **bispecific single chain antibody** mols. bearing specificities for the **human CD3** antigen and the human CD19 antigen are disclosed. These **bispecific single chain antibodies** are useful for treating proliferative disease, tumor, minimal residual cancer, inflammation, immune disease, autoimmune disease, infection, viral infection, parasitic infection, allergy, graft vs. host disease, and B cell malignancy.

L6 ANSWER 6 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN
2004:681433 Document No. 141:205678 Potent T cell modulating **bispecific** scFv constructs comprising modified VH-CDR3 region of anti-**human CD3 antibody**, OKT3, and therapeutic uses thereof. Lanzavecchia, Antonio (Micromet AG, Germany). U.S. Pat. Appl. Publ. US 2004162411 A1 20040819, 95 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-682845 20031010. PRIORITY: CA 2002-2403313 20021011; US 2002-2002/PV419149 20021018.

AB In accordance with the present invention it was found that a CDR3 region of an **antibody** mol., preferably directed against the CD3 on the surface of a T-cell, may be specifically modified. This specific modification(s)/mutation(s) as disclosed herein provide for modified **antibody** constructs as disclosed herein with altered physiol. and/or biochem. activities. The invention describes the use of **bispecific** scFv constructs comprising anti-human EpCAM x anti-**human CD3** for generation of mutants in the VH part of mouse anti-**human CD3** monoclonal **antibody** OKT3. The present invention describes **antibody** construct comprising at least one CDR3 region, wherein comprises at least one substitution in the amino acid sequence YYDDHY (SEQ ID NO.1). This at least one substitution comprises: in the first position of SEQ ID NO.1 a substitution from Y to H; in the second position a substitution from Y to S, from Y to N, from Y to F or from Y to H; in third position a substitution from D to N or from D to E; in the forth position of a substitution from D to Q, from D to A, from D to V, from D to E or from D to G; in the fifth position a substitution from H to Q, from H to P, from H to Y, from H to R or from H to N; or in the sixth position a substitution from Y to N.

L6 ANSWER 7 OF 21 MEDLINE on STN DUPLICATE 1
2004393790. PubMed ID: 15126676. Effect of linker sequences between the **antibody** variable domains on the formation, stability and biological activity of a **bispecific** tandem diabody. Le Gall Fabrice; Reusch Uwe; Little Melvyn; Kipriyanov Sergey M. (Affimed Therapeutics AG, Im Neuenheimer Feld 582, D-69120 Heidelberg, Germany.) Protein engineering, design & selection : PEDS, (2004 Apr) Vol. 17, No. 4, pp. 357-66. Electronic Publication: 2004-05-04. Journal code: 101186484. ISSN: 1741-0126. Pub. country: England: United Kingdom. Language: English.

AB **Bispecific single-chain** Fv **antibodies** comprise four covalently linked immunoglobulin variable (V(H) and V(L)) domains of two different specificities connected by three linkers. When assembled in the order V(H)(A)-linker(1)-V(L)(B)-linker(2)-V(H)(B)-linker(3)-V(L)(A), the **single-chain** molecule either folds head-to-tail with the formation of a diabody-like structure, a so-called **bispecific single-chain** diabody, or forms a homodimer that is twice as large, a so-called tandem diabody. The formation of the tandem diabody is determined by the association of complementary V(H) and V(L) domains located on different polypeptide chains, and depends on the length and probably the amino acid composition of the three linkers joining the variable domains. We generated a number of **single-chain** constructs using four V(H) and V(L) domains specific either for **human CD3**, a component of T-cell receptor (TCR) complex, or for CD19, a human B-cell antigen, separated by different rationally designed peptide linkers of 6-27 amino acid residues. The generated **bispecific** constructs were expressed in bacterial periplasm and their molecular forms,

antigen-binding properties, stability, and T-cell proliferative and anti-tumor activities were compared. Using peripheral blood mononuclear cell cultures from patients suffering from B-cell chronic lymphocytic leukemia, we demonstrated that the tandab-mediated activation of autologous T cells and depletion of malignant cells correlates with the stability of the recombinant molecule and with the distance between the CD19 and CD3 binding sites.

L6 ANSWER 8 OF 21 MEDLINE on STN DUPLICATE 2

2004050689. PubMed ID: 14751149. Characterization of an anti-human ovarian carcinomaxanti-**human CD3 bispecific single-chain antibody** with an albumin-original interlinker. Fang Min; Zhao Rui; Yang Zhi; Zhang Zhong; Li Hua; Zhang Xue-Tao; Lin Qing; Huang Hua-Liang. (Group 102, Institute of Genetics and Developmental Biology, Academia Sinica, Beijing 100101, China.) Gynecologic oncology, (2004 Jan) Vol. 92, No. 1, pp. 135-46. Journal code: 0365304. ISSN: 0090-8258. Pub. country: United States. Language: English.

AB OBJECTIVE: The objective of this study was to determine the properties of a **single-chain bispecific antibody** (scBsAb) against human ovarian carcinoma and to develop this agent for potential use in human ovarian cancer. METHODS: ELISA and FACS were performed to determine the antigen-binding properties of the scBsAb. Its abilities to retarget the pre-activated human peripheral blood mononuclear cells (PBMCs) to human ovarian carcinoma cell line SKOV3 cells and mediate their lysis in vitro were performed by a colorimetric MTT-based assay. Nude mice bearing human SKOV3 tumor xenografts were used to study the distribution and imaging of the scBsAb. Its pharmacokinetics in vivo was also studied in naive BALB/c mice. RESULTS: The scBsAb showed nearly identical ligand binding properties at each site relative to the individual monovalent **single-chain antibody** prototype molecules and could bridge SKOV3 and human T cell line Jurkat, which expresses CD3 antigens on the surface of cells together. It can also retarget the pre-activated PBMCs to SKOV3 cells and mediated their lysis in vitro effectively. Imaging and distribution study demonstrated that the **antibody** could target the tumor. Its elimination in vivo corresponded to second-order kinetics with a terminal half-life time (t(1/2)beta) of 7.7 h. CONCLUSION: This scBsAb with easy production and reasonable blood retention time should be developed for potential use in human ovarian cancer.

L6 ANSWER 9 OF 21 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN DUPLICATE 3

2003:1022545 The Genuine Article (R) Number: 742TG. Effects of interlinker sequences on the biological properties of **bispecific single-chain antibodies.** Fang M; Jiang X; Yang Z; Yin C C; Li H; Zhao R; Zhang Z; Lin Q; Huang H L (Reprint). Acad Sinica, Inst Genet & Dev Biol, Beijing 100101, Peoples R China (Reprint); Peking Univ, Sch Oncol, Beijing 100036, Peoples R China; Beijing Canc Hosp, Beijing 100036, Peoples R China. CHINESE SCIENCE BULLETIN (NOV 2003) Vol. 48, No. 21, pp. 2277-2283. ISSN: 1001-6538. Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Single-chain bispecific antibody** (scBsAb) is one of the promising genetic engineering **antibody** formats for clinical application. But the effects of interlinker sequences on the biological properties of **bispecific single-chain antibodies** have not been studied in deta it. Three interlinker sequences were designed and synthesized, and denominated as Fc, HSA, 205C', respectively. Universal vectors with these different interlinker sequences for scBsAb expression in E. coli were constructed. A model scBsAb based on a reshaped **single-chain antibody** (scFv) against **human CD3** and a scFv directed against human ovarian carcinoma were

generated and expressed in E. coli. The results of SDS-PAGE and Western blot showed that the different interlinker sequences did not affect the expression level of scBsAb. However, as demonstrated by ELISA and pharmacokinetics studies performed in juice, scBsAbs with different interlinker sequences had difference in the antigen-binding activities and terminal half-life time (T-1/2beta) in vivo, the interlinker HSA could remarkably prolong the retention time of scBsAb in blood. These results indicated that the peptide sequence of interlinker could affect important biological properties of scBsAb, such as antigen-binding properties and stability ill vivo. So, selection of an appropriate interlinker sequence is very important for scBsAb construction. Optimal interlinker can bring scBsAb biological properties more suitable for clinical application.

L6 ANSWER 10 OF 21 MEDLINE on STN DUPLICATE 4

2003550028. PubMed ID: 14628677. Production of soluble and functional engineered **antibodies** in Escherichia coli improved by FkpA. Zhang Zhong; Song Li-ping; Fang Min; Wang Fei; He Dan; Zhao Rui; Liu Jing; Zhou Zhi-yong; Yin Chang-cheng; Lin Qing; Huang Hua-liang. (Academia Sinica, Beijing, China.) BioTechniques, (2003 Nov) Vol. 35, No. 5, pp. 1032-8, 1041-2. Journal code: 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB Overproduction of genetically engineered **antibodies**, such as **single-chain antibodies** (scAbs) in Escherichia coli often results in insoluble and inactive products known as inclusion bodies. We now report that fusion or co-expression of FkpA, the E. coli periplasmic peptidyl-prolyl-isomerase with chaperone activity, substantially improves soluble and functional expression of scAbs. Anti-human bladder carcinoma scAb (PG) and anti-**human CD3** x anti-human ovarian carcinoma-**bispecific** scAb (BH1) were fused with FkpA on the pTMF-based plasmid and expressed in E. coli. More than half of the amount of each expressed fusion protein FkpA-PG or FkpA-BH1 was soluble. In addition, the fusion protein cellulose-binding domain from Cellulomonas fimi (CBD)-PG and anti-**human CD3** x anti-human CD28 x anti-human ovarian carcinoma-trispecific scAb (TRI) fused to the pelB (a signal peptide from pectate lysase B of a Bacillus sp.) signal sequence were co-expressed with FkpA under the control of the T7 promoter. A substantial portion of the co-expressed CBD-PG or TRI was soluble. Furthermore, PG, BH1, and TRI were biologically active as judged by ELISA and in vitro cytotoxicity assay. These results suggest that overexpression of FkpA should be useful in expressing heterologous proteins in E. coli.

L6 ANSWER 11 OF 21 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2003:561157 The Genuine Article (R) Number: 693XZ. Effect of domain order on the activity of bacterially produced **bispecific single -chain** Fv **antibodies.** Kipriyanov S M (Reprint); Moldenhauer G; Braunagel M; Reusch U; Cochlovius B; Le Gall F; Kouprianova O A; Von der Lieth C W; Little M. DKFZ, German Canc Res Ctr, Recombinant Antibody Res Grp, Neuenheimer Feld 280, D-69120 Heidelberg, Germany (Reprint); DKFZ, German Canc Res Ctr, Recombinant Antibody Res Grp, D-69120 Heidelberg, Germany; Affimed Therapeut AG, D-69120 Heidelberg, Germany; DKFZ, German Canc Res Ctr, Dept Mol Immunol, D-69120 Heidelberg, Germany; German Canc Res Ctr, Dept Spect, DKFZ, D-69120 Heidelberg, Germany. JOURNAL OF MOLECULAR BIOLOGY (27 JUN 2003) Vol. 330, No. 1, pp. 99-111. ISSN: 0022-2836. Publisher: ACADEMIC PRESS LTD ELSEVIER SCIENCE LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Bispecific single-chain** Fv **antibodies** comprise four covalently linked immunoglobulin variable (VH and VL) domains of two different specificities. Depending on the order of the VH and VL domains and on the length of peptides separating them, the **single-chain** molecule either forms two **single-chain** Fv (scFv) modules from the adjacent domains of the same specificity, a so-called scFv-scFv tandem [(scFv)2], or folds

head-to-tail with the formation of a diabody-like structure, a so-called **bispecific single-chain** diabody (scBsDb). We generated a number of four-domain constructs composed of the same VH and VL domains specific either for human CD19 or CD3, but arranged in different orders. When expressed in bacteria, all (scFv), variants appeared to be only half-functional, binding to CD19 and demonstrating no CD3-binding activity. Only the diabody-like scBsDb could bind both antigens. Comparison of the scBsDb with a structurally similar non-covalent dimer (diabody) demonstrated a stabilizing effect of the linker in the middle of the scBsDb molecule. We demonstrated that the mechanism of inactivation of CD19 X CD3 diabody under physiological conditions is initiated by a dissociation of the weaker (anti-CD3) VH/VL interface followed by domain swapping with the formation of non-active homodimers. The instability of one homodimer makes the process of diabody dissociation/reassociation irreversible, thus gradually decreasing the fraction of active molecules. The structural parameters influencing the formation of functional **bispecific single-chain antibodies** are indicated and ways of making relatively stable **bispecific** molecules are proposed.

L6 ANSWER 12 OF 21 MEDLINE on STN DUPLICATE 5

2002626155. PubMed ID: 12385030. Optimizing anti-CD3 affinity for effective T cell targeting against tumor cells. Bortoletto Nicola; Scotet Emmanuel; Myamoto Yoichi; D'Oro Ugo; Lanzavecchia Antonio. (IRIS, Chiron S.p.A., Siena, Italy.) European journal of immunology, (2002 Nov) Vol. 32, No. 11, pp. 3102-7. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB **Bispecific antibodies** binding to the TCR/CD3 complex and to a tumor-associated surface molecule can be used to target cytotoxic T lymphocytes against tumor cells. We reasoned that high-affinity binding to CD3 may reduce the efficiency of T cell stimulation and target the **bispecific** reagent to T cells rather than to tumor cells in vivo. We therefore mutated a **bispecific single-chain antibody** (BscAb) directed to **human CD3** and EpCAM to generate variants that bind to CD3 with higher or lower affinity. When compared to the wild-type molecule, a mutant with increased binding to CD3 showed lower capacity to target T cells against an EpCAM+ tumour. In contrast, mutants with decreased binding to CD3, in spite of rapid dissociation, efficiently triggered T cell activation and cytotoxicity, especially when present on tumor cells at low copy number. These results are consistent with the TCR serial triggering model and suggest that BscAb with extremely low affinity for the TCR-CD3 complex could be exploited therapeutically because of their preferential localization to tumor cells.

L6 ANSWER 13 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN

2003:538746 Document No. 139:394621 Construction and expression of an anti-human ovarian carcinoma x anti-**human CD3** **bispecific single-chain antibody** and its refolding studies. Fang, Min; Zhao, Rui; Li, Hua; Jiang, Xin; Yin, Changcheng; Lin, Qing; Huang, Hualiang (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101, Peop. Rep. China). Gaojishu Tongxun, 12(11), 47-50 (Chinese) 2002. CODEN: GTONE8. ISSN: 1002-0470. Publisher: Gaojishu Tongxun Zazhishe.

AB The anti-human ovarian carcinoma and anti-**human CD3** **bispecific single-chain antibody** (scBsAb) was constructed and expressed in the E.coli strain G1,724 as inclusion body. Three different refolding procedures were used to perform renaturation of the scBsAb. Direct dilution and gradual dialysis both caused the aggregation of the refolded proteins, however, size-exclusion chromatog. performing renaturation could suppress protein aggregation. ELISA tests showed that the refolded scBsAb had good activity, which can specifically bind to its target antigens.

L6 ANSWER 14 OF 21 MEDLINE on STN DUPLICATE 6

2000425985. PubMed ID: 10969772. Cure of Burkitt's lymphoma in severe combined immunodeficiency mice by T cells, tetravalent CD3 x CD19 tandem diabody, and CD28 costimulation. Cochlovius B; Kipriyanov S M; Stassar M J; Schuhmacher J; Benner A; Moldenhauer G; Little M. (Recombinant Antibody Research Group, German Cancer Research Center (DKFZ), Heidelberg.) Cancer research, (2000 Aug 15) Vol. 60, No. 16, pp. 4336-41. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB To increase the valency, stability, and therapeutic potential of **bispecific antibodies**, we have constructed a tetravalent tandem diabody (Tandab) that is specific to both **human CD3** (T-cell antigen) and CD19 (B-cell marker; S. M. Kipriyanov et al., J. Mol. Biol., 293: 41-56, 1999). It was generated by the functional dimerization of a **single chain** molecule that contained four **antibody** variable domains (V(H) and V(L)) in an orientation that prevented intramolecular pairing. Compared with a previously constructed heterodimeric CD3 x CD19 diabody, the Tandab exhibited a higher apparent affinity to both CD3+ and CD19+ cells and longer blood retention when injected into mice. Biodistribution studies in mice bearing Burkitt's lymphoma xenografts demonstrated specific accumulation of the radioiodinated Tandab in a tumor site with tumor-to-blood ratios of 1.5, 8.1, and 13.3 at 3, 18, and 24 h, respectively. Treatment of severe combined immunodeficiency mice bearing established Burkitt's lymphoma (5 mm in diameter) with human peripheral blood lymphocytes, Tandab, and anti-CD28 MAbs resulted in the complete elimination of tumors in all of the animals within 10 days. In contrast, mice receiving human peripheral blood lymphocytes in combination with either the diabody alone or the diabody plus anti-CD28 MAbs showed only partial tumor regression. These data demonstrate that the CD3 x CD19 Tandab may be a promising tool for the immunotherapy of human B-cell leukemias and lymphomas.

L6 ANSWER 15 OF 21 MEDLINE on STN DUPLICATE 7

1999443899. PubMed ID: 10512714. **Bispecific** tandem diabody for tumor therapy with improved antigen binding and pharmacokinetics. Kipriyanov S M; Moldenhauer G; Schuhmacher J; Cochlovius B; Von der Lieth C W; Matys E R; Little M. (Recombinant Antibody Research Group (D0500), German Cancer Research Center (DKFZ), Heidelberg.. s.kipriyanov@dkfz-heidelberg.de) . Journal of molecular biology, (1999 Oct 15) Vol. 293, No. 1, pp. 41-56. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB To increase the valency, stability and therapeutic potential of **bispecific antibodies**, we designed a novel recombinant molecule that is **bispecific** and tetravalent. It was constructed by linking four **antibody** variable domains (VHand VL) with specificities for **human CD3** (T cell antigen) or CD19 (B cell marker) into a **single chain** construct. After expression in Escherichia coli, intramolecularly folded bivalent **bispecific antibodies** with a mass of 57 kDa (**single chain** diabodies) and tetravalent **bispecific** dimers with a molecular mass of 114 kDa (tandem diabodies) could be isolated from the soluble periplasmic extracts. The relative amount of tandem diabodies proved to be dependent on the length of the linker in the middle of the chain and bacterial growth conditions. Compared to a previously constructed heterodimeric CD3xCD19 diabody, the tandem diabodies exhibited a higher apparent affinity and slower dissociation from both CD3(+)and CD19(+)cells. They were also more effective than diabodies in inducing T cell proliferation in the presence of tumor cells and in inducing the lysis of CD19(+)cells in the presence of activated human PBL. Incubated in human serum at 37 degrees C, the tandem diabody retained 90 % of its antigen binding activity after 24 hours and 40 % after one week. In vivo experiments indicated a higher stability and longer blood retention of tandem diabodies compared to **single chain** Fv fragments and diabodies, properties that are particularly important for potential clinical applications.

L6 ANSWER 16 OF 21 CAPLUS COPYRIGHT 2006 ACS on STN

1998:657018 Document No. 130:23896 Specific targeting of cytokine-secreting cells: a **bispecific** diabody recognizing human interleukin-6 and CD3 induces T cell-mediated killing. Krebs, Barbara; Ackermann, Birgit; Rose-John, Stefan (Dep. Med., Sect. Pathophysiol., Johannes Gutenberg-Univ., Mainz, D-55101, Germany). Journal of Interferon and Cytokine Research, 18(9), 783-791 (English) 1998. CODEN: JICRFJ. ISSN: 1079-9907. Publisher: Mary Ann Liebert, Inc..

AB Cytokines have been implicated in the pathophysiol. of many diseases. Although there have been many attempts to neutralize the activity of cytokines in vivo and in vitro, no strategies have been developed to specifically eliminate cells that overexpress cytokines. Considering that fact that cytokines in part remain cell associated on secretion, the authors have constructed a **bispecific** diabody consisting of non-neutralizing scFv **antibody** recognizing human interleukin-6 (IL-6) and an scFv corresponding to the monoclonal **antibody** (mAb) OKT3, which recognizes and activates the human T cell receptor. Here the authors show that the diabody recognized both human IL-6 and **human CD3.** In the presence of human T cells, the diabody induced killing of human hepatoma cells that had been transfected with a human IL-6 cDNA. The extent of killing was dependent on the ratio of effector/target cells in increased with increasing concns. of the diabody. Untransfected control cells or human hepatoma cells that secrete the IL-6-related cytokine leukemia inhibitory factor (LIF) remained unaffected. Thus, diabodies recognizing cytokines can be used to specifically target cytokine-secreting cells.

L6 ANSWER 17 OF 21 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

1997:448765 The Genuine Article (R) Number: XD510. Two amino acid mutations in an anti-**human CD3 single chain** Fv **antibody** fragment that affect the yield on bacterial secretion but not the affinity. Kipriyanov S M (Reprint); Moldenhauer G; Martin A C R; Kupriyanova O A; Little M. GERMAN CANC RES CTR DKFZ, RECOMBINANT ANTIBODY RES GRP 0445, DIAGNOST & EXPT THERAPY PROGRAMME, D-69120 HEIDELBERG, GERMANY; GERMAN CANC RES CTR DKFZ, TUMOR IMMUNOL PROGRAMME, DEPT MOL IMMUNOL 0740, D-69120 HEIDELBERG, GERMANY; UNIV LONDON UNIV COLL, DEPT BIOCHEM & MOL BIOL, BIOMOL STRUCT & MODELING UNIT, LONDON WC1E 6BT, ENGLAND. PROTEIN ENGINEERING (APR 1997) Vol. 10, No. 4, pp. 445-453. ISSN: 0269-2139. Publisher: OXFORD UNIV PRESS, GREAT CLARENDON ST, OXFORD, ENGLAND OX2 6DP. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Recombinant **antibody** fragments directed against cell surface antigens have facilitated the development of novel therapeutic agents. As a first step in the creation of cytotoxic immunoconjugates, we constructed a **single-chain** Fv fragment derived from the murine hybridoma OKT3, that recognizes an epitope on the epsilon-subunit of the **human CD3** complex. Two amino acid residues were identified that are critical for the high level production of this scFv in Escherichia coil, First, the substitution of glutamic acid encoded by a PCR primer at position 6 of V-H framework 1 by glutamine led to a more than a 30-fold increase in the production of soluble scFv, Second, the substitution of cysteine by a serine in the middle of CDR-H3 additionally doubled the yield of soluble **antibody** fragment without any adverse effect on its affinity for the CD3 antigen, The double mutant scFv (Q,S) proved to be very stable in vitro: no loss of activity was observed after storage for 1 month at 4 degrees C, while the activity of scFv containing a cysteine residue in CDR-H3 decreased by more than half. The results of production yield, affinity, stability measurements and analysis of three-dimensional models of the structure suggest that the sixth amino acid influences the correct folding of the V-H domain, presumably by affecting a folding intermediate, but has no effect on antigen binding.

L6 ANSWER 18 OF 21 MEDLINE on STN DUPLICATE 8
97214920. PubMed ID: 9061285. Mono- and **bispecific** **single-chain antibody** fragments for cancer therapy. Thirion S; Motmans K; Heyligen H; Janssens J; Raus J; Vandevyver C. (Dr L Willems-Instituut, Diepenbeek, Belgium.) European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP), (1996 Dec) Vol. 5, No. 6, pp. 507-11. Journal code: 9300837. ISSN: 0959-8278. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Especially when dealing with solid cancers, **single-chain** **antibody** fragments (scFvs) have a lot of advantages. Due to their small size (27 kDa), these proteins clear more rapidly from the blood, and penetrate faster and deeper into tissues, than whole **antibodies.** Furthermore, the lack of constant regions ensures that they are not retained in tissues such as the liver and kidney. This reduces possible toxic side-effects. **Single-chain** construction is normally done by polymerase chain reaction (PCR). To decrease the overall cost of oligonucleotide primer synthesis, time-consuming primer design, multiple PCR reactions and individual PCR optimization, we designed a universal single-step overlap extension PCR protocol using hybridoma cDNA as a template. To overcome the lack of effector function, **bispecific** scFvs, consisting of an scFv produced against a tumour-associated antigen fused to a T cell marker-specific scFv, are being created, starting from already assembled scFv, by means of two additional PCR reactions. In this paper we describe both PCR methods that were successfully used to create scFvs against the human transferrin receptor, the human interleukin-2 receptor, the **human** **CD3** molecule, a breast tumour-associated antigen and an anti-transferrin-anti-CD3 **bispecific** scFv.

L6 ANSWER 19 OF 21 MEDLINE on STN DUPLICATE 9
96053283. PubMed ID: 7584477. **Single-chain** mono- and **bispecific antibody** derivatives with novel biological properties and antitumour activity from a COS cell transient expression system. Hayden M S; Linsley P S; Gayle M A; Bajorath J; Brady W A; Norris N A; Fell H P; Ledbetter J A; Gilliland L K. (Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Autoimmunity and Transplantation, Seattle, WA 98121, USA.) Therapeutic immunology, (1994 Jan) Vol. 1, No. 1, pp. 3-15. Journal code: 9421528. ISSN: 0967-0149. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Single-chain antibody** molecules were expressed from modified eukaryotic expression vectors as individual protein domains encoded on interchangeable cDNA cassettes. Two different **single-chain antibody** derivatives were constructed by linking individual light- and heavy-chain variable domains. The first was specific for the L6 tumour-associated antigen and the second was specific for **human CD3.** Each **single-** **chain** variable domain was genetically fused with an Fc 'tag' and expressed as a fusion protein in a COS cell transient transfection system. These **single-chain antibody** derivatives demonstrated specific binding to cells expressing appropriate antigen and bound with affinities similar to native **antibody.** The CD3 **single chain** molecule mediated stronger activation of PLC gamma 1 and similar levels of T-cell proliferation compared with native **antibody.** A **bispecific** Fv **single-** **chain** cassette was created by fusing the expression cassettes encoding the binding domains for L6 and CD3 **single-chain** molecules using oligonucleotide primers encoding a short 27-residue 'helical' peptide linker. The CD3-L6 variable domains were fused to the Fc tag and expressed in COS cells. The CD3-L6FvIg **bispecific** fusion protein mediated adhesion between T cells and L6-positive tumour cells, and stimulated potent T-cell proliferation and cytotoxicity against tumour cells expressing the L6 antigen.

L6 ANSWER 20 OF 21 MEDLINE on STN DUPLICATE 10

93053064. PubMed ID: 1428404. Janusin: new molecular design for **bispecific** reagents. Traunecker A; Lanzavecchia A; Karjalainen K. (Basel Institute for Immunology, Switzerland.) International journal of cancer. Supplement = Journal international du cancer. Supplement, (1992) Vol. 7, pp. 51-2. Ref: 19. Journal code: 8710267. ISSN: 0898-6924. Pub. country: United States. Language: English.

AB It is well established that soluble CD4 (sCD4) inhibits HIV infection in vitro, regardless of the virus strain or genetic variant. Most effective molecules, thus far, based on sCD4 are those in which CD4 is combined with immunoglobulin constant regions (CD4-IgG or CD4-IgM). Such molecules maintained HIV-gp120 specificity mediated by CD4 and also **antibody** effector functions such as complement activation, Fc receptor binding, long serum half-life or transport across the placental barrier. We have now developed sCD4 molecules which are even more potent anti-HIV reagents. These molecules are based on the principle of **bispecific antibodies** and they have properties capable of retargeting cytotoxic T lymphocytes onto HIV-infected cells and inducing efficient killing. CD4 combined with anti-**human CD3** (FvCD3) **single-chain** combining site has been produced (CD4-FvCD3-JANUSIN). This molecule shows the expected biological activities, namely, binding to the 2 ligands, **human CD3** and gp120, also efficiently retargeting CTLs of any specificity onto HIV-infected cells. In addition, several advantages over classical **bispecific antibodies** can be achieved: only one polypeptide, not a mixture containing the desired product, is produced, thus simplifying the purification process. In addition, Janusin designs do not contain the Ig Fc portion, which could mediate illegitimate retargeting of T-cells. In addition to CD4-FvCD3-JANUSIN, receptor-Fv, Fv-Fv or ligand-Fv Janusins can be produced.

L6 ANSWER 21 OF 21 MEDLINE on STN DUPLICATE 11

92037526. PubMed ID: 1834458. **Bispecific single chain** molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells. Traunecker A; Lanzavecchia A; Karjalainen K. (Basel Institute for Immunology, Switzerland.) The EMBO journal, (1991 Dec) Vol. 10, No. 12, pp. 3655-9. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The human immunodeficiency virus type 1 (HIV-1) uses cell surface CD4 as a receptor to infect susceptible cells. Therefore, different forms of soluble CD4 (sCD4) molecules have been developed recently for potential therapeutic purposes. Here we describe a novel design of sCD4 molecules which exploit cytotoxic T cells as their effector function. The principle of **bispecific antibodies** was exploited and further developed to create new **bispecific** reagents which could retarget cytotoxic T cells of any specificity and thus, induce killing of HIV-1 infected cells. The most advanced molecules, Janusins, contain in one polypeptide chain the first two N-terminal CD4 domains and **single chain** combining site against the **human CD3** complex (FvCD3).

=> s l4 and tumor antigen 17-1A
L7 3 L4 AND TUMOR ANTIGEN 17-1A

=> dup remove l7
PROCESSING COMPLETED FOR L7
L8 3 DUP REMOVE L7 (0 DUPLICATES REMOVED)

=> d l8 1-3 cbib abs

L8 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN

2004:1059395 Document No. 142:36927 **Bispecific single chain antibodies** against human EpCAM and **human CD3** antigen for cancer therapy. Kufer, Peter; Berry, Meera; Offner, Sonja; Brischwein, Klaus; Wolf, Andreas; Raum, Tobias; Kohleisen,

Birgit; Lenkkeri-Schuetz, Ulla; Baeuerle, Patrick (Micromet A.-G., Germany). PCT Int. Appl. WO 2004106383 A1 20041209, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP5687 20040526. PRIORITY: EP 2003-12134 20030531; EP 2003-12133 20030531.

AB The present invention provides a pharmaceutical composition comprising a **bispecific single chain antibody** construct. Said **bispecific single chain antibody** construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to **human CD3** antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed **bispecific single chain antibody** construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

L8 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN

2004:681433 Document No. 141:205678 Potent T cell modulating **bispecific** scFv constructs comprising modified VH-CDR3 region of anti-**human CD3 antibody**, OKT3, and therapeutic uses thereof. Lanzavecchia, Antonio (Micromet AG, Germany). U.S. Pat. Appl. Publ. US 2004162411 A1 20040819, 95 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-682845 20031010. PRIORITY: CA 2002-2403313 20021011; US 2002-2002/PV419149 20021018.

AB In accordance with the present invention it was found that a CDR3 region of an **antibody** mol., preferably directed against the CD3 on the surface of a T-cell, may be specifically modified. This specific modification(s)/mutation(s) as disclosed herein provide for modified **antibody** constructs as disclosed herein with altered physiol. and/or biochem. activities. The invention describes the use of **bispecific** scFv constructs comprising anti-human EpCAM x anti-**human CD3** for generation of mutants in the VH part of mouse anti-**human CD3** monoclonal **antibody** OKT3. The present invention describes **antibody** construct comprising at least one CDR3 region, wherein comprises at least one substitution in the amino acid sequence YYDDHY (SEQ ID NO.1). This at least one substitution comprises: in the first position of SEQ ID NO.1 a substitution from Y to H; in the second position a substitution from Y to S, from Y to N, from Y to F or from Y to H; in third position a substitution from D to N or from D to E; in the forth position of a substitution from D to Q, from D to A, from D to V, from D to E or from D to G; in the fifth position a substitution from H to Q, from H to P, from H to Y, from H to R or from H to N; or in the sixth position a substitution from Y to N.

L8 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN

2002:899901 Document No. 137:368202 Optimizing anti-CD3 affinity for effective T cell targeting against tumor cells. Bortoletto, Nicola; Scotet, Emmanuel; Myamoto, Yoichi; D'Oro, Ugo; Lanzavecchia, Antonio (IRIS, Chiron S.p.A., Siena, Italy). European Journal of Immunology, 32(11), 3102-3107 (English) 2002. CODEN: EJIMAF. ISSN: 0014-2980. Publisher: Wiley-VCH Verlag GmbH & Co. KGaA.

AB **Bispecific antibodies** binding to the TCR/CD3 complex and to a tumor-associated surface mol. can be used to target cytotoxic T

lymphocytes against tumor cells. We reasoned that high-affinity binding to CD3 may reduce the efficiency of T cell stimulation and target the **bispecific** reagent to T cells rather than to tumor cells in vivo. We therefore mutated a **bispecific single-chain antibody** (BscAb) directed to **human CD3** and EpCAM to generate variants that bind to CD3 with higher or lower affinity. When compared to the wild-type mol., a mutant with increased binding to CD3 showed lower capacity to target T cells against an EpCAM+ tumor. In contrast, mutants with decreased binding to CD3, in spite of rapid dissociation, efficiently triggered T cell activation and cytotoxicity, especially when present on tumor cells at low copy number These results are consistent with the TCR serial triggering model and suggest that BscAb with extremely low affinity for the TCR-CD3 complex could be exploited therapeutically because of their preferential localization to tumor cells.

=> s l7 and 17-1A
L9 3 L7 AND 17-1A

=> d l9 1-3 cbib abs

L9 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
2004:1059395 Document No. 142:36927 **Bispecific single chain antibodies** against human EpCAM and **human CD3** antigen for cancer therapy. Kufer, Peter; Berry, Meera; Offner, Sonja; Brischwein, Klaus; Wolf, Andreas; Raum, Tobias; Kohleisen, Birgit; Lenkkeri-Schuetz, Ulla; Baeuerle, Patrick (Micromet A.-G., Germany). PCT Int. Appl. WO 2004106383 A1 20041209, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP5687 20040526. PRIORITY: EP 2003-12134 20030531; EP 2003-12133 20030531.

AB The present invention provides a pharmaceutical composition comprising a **bispecific single chain antibody** construct. Said **bispecific single chain antibody** construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to **human CD3** antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed **bispecific single chain antibody** construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

L9 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
2004:681433 Document No. 141:205678 Potent T cell modulating **bispecific** scFv constructs comprising modified VH-CDR3 region of anti-**human CD3 antibody**, OKT3, and therapeutic uses thereof. Lanzavecchia, Antonio (Micromet AG, Germany). U.S. Pat. Appl. Publ. US 2004162411 A1 20040819, 95 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-682845 20031010. PRIORITY: CA 2002-2403313 20021011; US 2002-2002/PV419149 20021018.

AB In accordance with the present invention it was found that a CDR3 region of an **antibody** mol., preferably directed against the CD3 on the surface of a T-cell, may be specifically modified. This specific

modification(s)/mutation(s) as disclosed herein provide for modified **antibody** constructs as disclosed herein with altered physiol. and/or biochem. activities. The invention describes the use of **bispecific** scFv constructs comprising anti-human EpCAM x anti-**human CD3** for generation of mutants in the VH part of mouse anti-**human CD3** monoclonal **antibody** OKT3. The present invention describes **antibody** construct comprising at least one CDR3 region, wherein comprises at least one substitution in the amino acid sequence YYDDHY (SEQ ID NO.1). This at least one substitution comprises: in the first position of SEQ ID NO.1 a substitution from Y to H; in the second position a substitution from Y to S, from Y to N, from Y to F or from Y to H; in third position a substitution from D to N or from D to E; in the forth position of a substitution from D to Q, from D to A, from D to V, from D to E or from D to G; in the fifth position a substitution from H to Q, from H to P, from H to Y, from H to R or from H to N; or in the sixth position a substitution from Y to N.

L9 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
2002:899901 Document No. 137:368202 Optimizing anti-CD3 affinity for effective T cell targeting against tumor cells. Bortoletto, Nicola; Scotet, Emmanuel; Myamoto, Yoichi; D'Oro, Ugo; Lanzavecchia, Antonio (IRIS, Chiron S.p.A., Siena, Italy). European Journal of Immunology, 32(11), 3102-3107 (English) 2002. CODEN: EJIMAF. ISSN: 0014-2980. Publisher: Wiley-VCH Verlag GmbH & Co. KGaA.
AB **Bispecific antibodies** binding to the TCR/CD3 complex and to a tumor-associated surface mol. can be used to target cytotoxic T lymphocytes against tumor cells. We reasoned that high-affinity binding to CD3 may reduce the efficiency of T cell stimulation and target the **bispecific** reagent to T cells rather than to tumor cells in vivo. We therefore mutated a **bispecific single-chain antibody** (BscAb) directed to **human CD3** and EpCAM to generate variants that bind to CD3 with higher or lower affinity. When compared to the wild-type mol., a mutant with increased binding to CD3 showed lower capacity to target T cells against an EpCAM+ tumor. In contrast, mutants with decreased binding to CD3, in spite of rapid dissociation, efficiently triggered T cell activation and cytotoxicity, especially when present on tumor cells at low copy number These results are consistent with the TCR serial triggering model and suggest that BscAb with extremely low affinity for the TCR-CD3 complex could be exploited therapeutically because of their preferential localization to tumor cells.

=> s (kuffer p?/au or berry m?/au or baeuerle p?/au)
L10 6418 (KUFFER P?/AU OR BERRY M?/AU OR BAEUERLE P?/AU)

=> s l10 and bispecific single chain antibody
L11 51 L10 AND BISPECIFIC SINGLE CHAIN ANTIBODY

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12 15 DUP REMOVE L11 (36 DUPLICATES REMOVED)

=> d l12 1-15 cbib abs

L12 ANSWER 1 OF 15 MEDLINE on STN DUPLICATE 1
2006027610. PubMed ID: 16139892. MT110: a novel **bispecific single-chain antibody** construct with high efficacy in eradicating established tumors. Brischwein Klaus; Schlereth Bernd; Guller Benjamin; Steiger Carola; Wolf Andreas; Lutterbuese Ralf; Offner Sonja; Locher Mathias; Urbig Thomas; Raum Tobias; Kleindienst Petra; Wimberger Pauline; Kimmig Rainer; Fichtner Iduna; Kufer Peter; Hofmeister Robert; da Silva Antonio J; **Baeuerle Patrick A.** (Micromet AG, Staffelseestr. 2, 81477 Munich, Germany.) Molecular

immunology, (2006 Mar) Vol. 43, No. 8, pp. 1129-43. Electronic Publication: 2005-09-01. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB We have developed a novel single-chain Ep-CAM-/CD3-**bispecific single-chain antibody** construct designated MT110. MT110 redirected unstimulated human peripheral T cells to induce the specific lysis of every Ep-CAM-expressing tumor cell line tested. MT110 induced a costimulation independent polyclonal activation of CD4- and CD8-positive T cells as seen by de novo expression of CD69 and CD25, and secretion of interferon gamma, tumor necrosis factor alpha, and interleukins 2, 4 and 10. CD8-positive T cells made the major contribution to redirected tumor cell lysis by MT110. With a delay, CD4-positive cells could also contribute presumably as consequence of a dramatic upregulation of granzyme B expression. MT110 was highly efficacious in a NOD/SCID mouse model with subcutaneously growing SW480 human colon cancer cells. Five daily doses of 1 microg MT110 on days 0-4 completely prevented tumor outgrowth in all mice treated. The bispecific antibody construct also led to a durable eradication of established tumors in all mice treated with 1 microg doses of MT110 on days 8-12 after tumor inoculation. Finally, MT110 could eradicate patient-derived metastatic ovarian cancer tissue growing under the skin of NOD/SCID mice. MT110 appears as an attractive bispecific antibody candidate for treatment of human Ep-CAM-overexpressing carcinomas.

L12 ANSWER 2 OF 15 MEDLINE on STN DUPLICATE 2

2005674238. PubMed ID: 16360021. Induction of regular cytolytic T cell synapses by **bispecific single-chain antibody** constructs on MHC class I-negative tumor cells. Offner Sonja; Hofmeister Robert; Romaniuk Andrea; Kufer Peter; **Baeuerle Patrick A.** (Micromet AG, Staffelseestr. 2, 81477 Munich, Germany.) Molecular immunology, (2006 Feb) Vol. 43, No. 6, pp. 763-71. Electronic Publication: 2005-04-26. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB Certain **bispecific single-chain antibody** constructs exhibit an extraordinary potency for polyclonal T cell engagement and target cell lysis. Here we studied the structural basis for this potency, using laser scanning confocal microscopy. Cytolytic human T cell synapses could be triggered either by addition of a specific peptide antigen or an Ep-CAM-/CD3-bispecific T cell engager (BiTE). Both kinds of synapses showed a comparable distribution of all protein markers investigated. Two other BiTEs constructed from different Ep-CAM-specific antibodies gave similar results. BiTEs could also induce lytic synapses between human T cells and a MHC class I-negative, Ep-CAM cDNA-transfected cell line resulting in potent target cell lysis. This shows that certain T cell recognition molecules on target cells are dispensable for synapse formation and BiTE activity, and suggests that BiTE-activated polyclonal T cells may ignore major immune evasion mechanisms of tumor cells in vivo, such as loss of MHC class I expression.

L12 ANSWER 3 OF 15 MEDLINE on STN DUPLICATE 3

2006150886. PubMed ID: 16032400. T-cell activation and B-cell depletion in chimpanzees treated with a bispecific anti-CD19/anti-CD3 single-chain antibody construct. Schlereth Bernd; Quadt Cornelia; Dreier Torsten; Kufer Peter; Lorenczewski Grit; Prang Nadja; Brandl Christian; Lippold Sandra; Cobb Kathy; Brasky Kathleen; Leo Eugen; Bargou Ralf; Murthy Krishna; **Baeuerle Patrick A.** (Micromet AG, Staffelseestr. 2, 81477, Munich, Germany.) Cancer immunology, immunotherapy : CII, (2006 May) Vol. 55, No. 5, pp. 503-14. Electronic Publication: 2005-07-20. Journal code: 8605732. ISSN: 0340-7004. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB BscCD19xCD3 is a **bispecific single-chain antibody** construct with exceptional cytotoxic potency in vitro and in vivo. Here, we have investigated the biological activity of bscCD19xCD3 in chimpanzee, the only animal species identified in which

bscCD19xCD3 showed bispecific binding, redirected B-cell lysis and cytokine production comparable to human cells. Pharmacokinetic analysis following 2-h intravenous infusion of 0.06, 0.1 or 0.12 mug/kg of bscCD19xCD3 as part of a dose escalation study in a single female chimpanzee revealed a half-life of approximately 2 h and elimination of the bispecific antibody from circulation within approximately 8 h after the end of infusion. This short exposure to bscCD19xCD3 elicited a transient increase in serum levels of IFNgamma, IL-6, IL-2, soluble CD25, and transiently upregulated expression of CD69 and MHC class II on CD8-positive cells. Cytokine release and upregulation of T-cell activation markers were not observed with vehicle controls. A multiple-dose study using 5 weekly doses of 0.1 mug/kg in two animals also showed transient cytokine release and an activation of peripheral T cells with a first-dose effect, accompanied by a transient lymphopenia. While oscillations of T-cell counts were relatively even during repeated treatments, the amplitudes of peripheral B cells declined with every infusion, which was not observed in a vehicle control animal. Our data show that bscCD19xCD3 can be safely administered to chimpanzees at dose levels that cause fully reversible T-cell activation and, despite a very short exposure time, cumulative loss of peripheral B lymphocytes. A clinical trial testing prolonged administration of bscCD19xCD3 (MT103) for improving efficacy is currently ongoing.

L12 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
2005:546858 Document No. 143:76820 **Bispecific single-chain antibodies** preparation and therapeutic uses thereof. Kufer, Peter; **Berry, Meera; Baeuerle, Patrick** ; Itin, Christian (Germany). U.S. Pat. Appl. Publ. US 2005136050 A1 20050623, 12 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-743697 20031222.

AB The present invention discloses bispecific antibodies comprising two antibody variable domains on a single polypeptide chain, wherein a first portion of the bispecific antibody is capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen on the human immune effector cell, the first portion consisting of one antibody variable domain, and a second portion of the bispecific antibody specifically binding to a target antigen other than the effector antigen, the target antigen on a target cell other than the human immune effector cell, the second portion comprising one antibody variable domain (or 2 antibody variable domains). The inventors describe preparation and purification of a bispecific antibody with 3 antibody variable domains located on the same polypeptide chain. Progressing from the N to C terminus, the antibody contains: anti-human EpCAM VL; 15 amino acid linker of sequence (Gly4Ser)3; anti-human EpCAM VH; 5 amino acid spacer Gly4Ser; anti-human CD3 VH; His6. This antibody showed activity as a recruiter of cytotoxic T cells as shown by the efficient killing of the target cells in a manner depending on the concentrate of the bispecific antibody added to a reaction mixture in the presence of cytotoxic T cells.

L12 ANSWER 5 OF 15 MEDLINE on STN DUPLICATE 4
2005172561. PubMed ID: 15805290. Eradication of tumors from a human colon cancer cell line and from ovarian cancer metastases in immunodeficient mice by a single-chain Ep-CAM-/CD3-bispecific antibody construct. Schlereth Bernd; Fichtner Iduna; Lorenczewski Grit; Kleindienst Petra; Brischwein Klaus; da Silva Antonio; Kufer Peter; Lutterbuese Ralf; Junghahn Ilse; Kasimir-Bauer Sabine; Wimberger Pauline; Kimmig Rainer; **Baeuerle Patrick A.** (Micromet AG, Munich, Germany.) Cancer research, (2005 Apr 1) Vol. 65, No. 7, pp. 2882-9. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Bispecific T-cell engager (BiTE) are a class of **bispecific single-chain antibodies** that can very effectively redirect cytotoxic T cells for killing of tumor target cells. Here, we have assessed the in vivo efficacy of one representative, called bscEp-CAMxCD3, with specificity for tumors overexpressing epithelial cell adhesion molecule (Ep-CAM) in human xenograft models. Cells of the human

colon carcinoma line SW480 were mixed at a 1:1 ratio with unstimulated human peripheral mononuclear cells, s.c. injected in nonobese diabetes/severe combined immunodeficiency (NOD/SCID) mice, and animals were treated with bscEp-CAMxCD3. Five daily i.v. injections of as little as 100 ng per mouse of bscEp-CAMxCD3 completely prevented tumor outgrowth when treatment was started at the day of tumor cell inoculation. BscEp-CAMxCD3 was also efficacious when administered up to 8 days after xenograft injection. Established tumors could be eradicated in all animals by five 10 microg doses given between days 8 and 12 after tumor cell inoculation. To test the efficacy of bscEp-CAMxCD3 in a more physiologic model, pieces of primary metastatic tumor tissue from ovarian cancer patients were implanted in NOD/SCID mice. Partial tumor engraftment and growth was observed with four of six patient samples. Treatment of established tumors with daily 5 microg doses led to a significant reduction and, in some cases, eradication of human tumor tissue. These effects obviously relied on the tumor-resident T cells reactivated by bscEp-CAMxCD3. Our data show that the class of single-chain bispecific antibodies has very high antitumor efficacy in vivo and can use previously unstimulated T cells at low effector-to-target ratios.

L12 ANSWER 6 OF 15 MEDLINE on STN DUPLICATE 5
2005536804. PubMed ID: 16213416. BiTEs: bispecific antibody constructs with unique anti-tumor activity. Wolf Evelyn; Hofmeister Robert; Kufer Peter; Schlereth Bernd; **Baeuerle Patrick A.** (Micromet AG, Staffelseestr. 2, 81477 Munich, Germany.) Drug discovery today, (2005 Sep 15) Vol. 10, No. 18, pp. 1237-44. Ref: 54. Journal code: 9604391. ISSN: 1359-6446. Pub. country: England: United Kingdom. Language: English.
AB Bispecific T-cell engager molecules (BiTEs) constitute a class of **bispecific single-chain antibodies** for the polyclonal activation and redirection of cytotoxic T cells against pathogenic target cells. BiTEs combine a unique set of properties that have not yet been reported for any other kind of bispecific antibody construct, namely extraordinary potency and efficacy against target cells at low T-cell numbers without the need for T-cell co-stimulation. Here we review novel insights into the mechanism of BiTE action, which help to explain the unique features of BiTEs, as well as data from various animal models demonstrating the outstanding therapeutic potential of BiTEs for the treatment of malignant diseases.

L12 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN
2005:1328352 Bispecific T cell-recruiting antibody constructs for cancer therapy. **Baeuerle, Patrick A.** (Micromet AG, Munich, Germany). Arzneimittel Forschung, 55(11), 697-698 (English) 2005. CODEN: ARZNAD. ISSN: 0004-4172. Publisher: Editio Cantor Verlag.
AB Monoclonal antibody therapies show great promise for the treatment of cancer. This stimulated the search for other targeted therapies that may increase the cytotoxic potential of monoclonal antibodies. One approach is the temporary conjugation of target (tumor cells) with cytotoxic immune cells using bispecific antibodies. Bispecific T cell engager (BiTE) mols. comprise a class of **bispecific single-chain antibodies** for the polyclonal activation and re-direction of cytotoxic T cells against pathogenic target cells. Ex-vivo expts. and many in-vivo models have consistently shown a high anti-tumor activity of BiTEs. MT103, a CD19-specific BiTE, is currently in a dose-escalating phase I trial.

L12 ANSWER 8 OF 15 MEDLINE on STN DUPLICATE 6
2005160513. PubMed ID: 15688411. Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-**bispecific single-chain antibody** construct. Hoffmann Patrick; Hofmeister Robert; Brischwein Klaus; Brandl Christian; Crommer Sandrine; Bargou Ralf; Itin Christian; Prang Nadja; **Baeuerle Patrick A.** (Micromet AG, Munich, Germany.) International journal of cancer. Journal international du cancer, (2005 May 20) Vol. 115, No. 1, pp.

98-104. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Certain bispecific antibodies exhibit an extraordinary potency and efficacy for target cell lysis by eliciting a polyclonal T-cell response. One example is a CD19-/CD3-**bispecific single-chain antibody** construct (bscCD19xCD3), which at femtomolar concentrations can redirect cytotoxic T cells to eliminate human B lymphocytes, B lymphoma cell lines and patient-derived malignant B cells. Here we have further explored the basis for this high potency. Using video-assisted microscopy, bscCD19xCD3 was found to alter the motility and activity of T cells from a scanning to a killing mode. Individual T cells could eliminate multiple target cells within a 9 hr time period, resulting in nuclear fragmentation and membrane blebbing of target cells. Complete target cell elimination was observed within 24 hr at effector-to-target cell ratios as low as 1:5. Under optimal conditions, cell killing started within minutes after addition of bscCD19xCD3, suggesting that the rate of serial killing was mostly determined by T-cell movement and target cell scanning and lysis. At all times, T cells remained highly motile, and no clusters of T and target cells were induced by the bispecific antibody. Bystanding target-negative cells were not detectably affected. Repeated target cell lysis by bscCD19xCD3-activated T cells increased the proportion of CD19/CD3 double-positive T cells, which was most likely a consequence of transfer of CD19 from B to T cells during cytolytic synapse formation. To our knowledge, this is the first study showing that a bispecific antibody can sustain multiple rounds of target cell lysis by T cells.

L12 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

2004:1059395 Document No. 142:36927 **Bispecific single chain antibodies** against human EpCAM and human CD3 antigen for cancer therapy. Kufer, Peter; **Berry, Meera**; Offner, Sonja; Brischwein, Klaus; Wolf, Andreas; Raum, Tobias; Kohleisen, Birgit; Lenkkeri-Schuetz, Ulla; **Baeuerle, Patrick** (Micromet A.-G., Germany). PCT Int. Appl. WO 2004106383 A1 20041209, 227 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP5687 20040526. PRIORITY: EP 2003-12134 20030531; EP 2003-12133 20030531.

AB The present invention provides a pharmaceutical composition comprising a **bispecific single chain antibody** construct. Said **bispecific single chain antibody** construct is characterized to comprise or consist of at least two domains, whereby one of said at least two domains specifically binds to human EpCAM and comprises at least one CDR-H3 region comprising the amino acid sequence NXID antigen and a second domain binds to human CD3 antigen. The invention further provides a process for the production of the pharmaceutical composition of the invention, a method for the prevention, treatment or amelioration of a tumorous disease and the use of the disclosed **bispecific single chain antibody** construct and corresponding means in the prevention, treatment or amelioration of a tumorous disease.

L12 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2006 ACS on STN

2004:1059393 Document No. 142:36925 Pharmaceutical compositions comprising bispecific anti-human CD3, anti-human CD19 single chain antibodies for treating B-cell related disorders. Kufer, Peter; Lutterbuese, Ralf; Kohleisen, Birgit; Zeman, Steven; **Baeuerle, Patrick** (Micromet A.-G., Germany). PCT Int. Appl. WO 2004106381 A1 20041209, 115 pp.

DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2004-EP5685 20040526. PRIORITY: EP 2003-12136 20030531.

AB The present invention relates to a pharmaceutical composition comprising a **bispecific single chain antibody** construct, said **bispecific single chain antibody** construct comprising binding domains specific for human CD3 and human CD19, wherein the corresponding variable heavy chain regions (VH) and the corresponding variable light chain regions (VL) regions are arranged, from N-terminus to C-terminus, in the order, VH(CD19)-VL(CD19)-VH(CD3)-VL(CD3), VH(CD3)-VL-(CD3)-VH(CD19)-VL(CD19) or VH(CD3)-VL(CD3)-VL(CD19)-VH(CD19). Furthermore, processes for the production of said pharmaceutical compns. as well as medical/pharmaceutical uses for the specific **bispecific single chain antibody** mols. bearing specificities for the human CD3 antigen and the human CD19 antigen are disclosed. These **bispecific single chain antibodies** are useful for treating proliferative disease, tumor, minimal residual cancer, inflammation, immune disease, autoimmune disease, infection, viral infection, parasitic infection, allergy, graft vs. host disease, and B cell malignancy.

L12 ANSWER 11 OF 15 MEDLINE on STN DUPLICATE 7

2004284415. PubMed ID: 15183929. A **bispecific single-chain antibody** fusion protein for targeted depletion of autoreactive B cells via unstimulated human T lymphocytes. Zocher Marcel; **Baeuerle Patrick A.** (Micromet AG, Staffelseestr. 2, 81477 Munich, Germany.. marcel.zocher@roche.com) . Molecular immunology, (2004 Jul) Vol. 41, No. 5, pp. 511-8. Journal code: 7905289. ISSN: 0161-5890. Pub. country: England: United Kingdom. Language: English.

AB Autoantigen-specific B cells are culprits in the pathogenesis of many autoimmune diseases either through the production of autoreactive antibodies or as very effective antigen-presenting cells. A general depletion of B cells by a CD20-specific monoclonal IgG1 antibody has recently been validated as an effective strategy for treating rheumatoid arthritis. However, general elimination of B cells can lead to immunosuppression and increased risk of infection. In search for a more specific approach, we have generated a fusion protein for the antigen-specific targeting of autoreactive B cells for re-directed lysis by resting human T lymphocytes. We describe the design, purification and characterization of MOGxanti-CD3, a single-chain bispecific antibody fusion protein recognizing B cell receptors specific for the human myelin oligodendrocyte glycoprotein (MOG) and to CD3 on human T cells. MOGxAnti-CD3 induced selective and very efficient redirected lysis of MOG-reactive B cells through freshly isolated, unstimulated human T cells. Fusions between autoantigens and an anti-CD3 single-chain antibody may be suitable to develop very specific therapeutic approaches for the selective depletion of autoreactive B cells in autoimmune diseases.

L12 ANSWER 12 OF 15 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

2004:51146 The Genuine Article (R) Number: 756LU. High efficacy of a **bispecific single-chain antibody** construct (bscCD19xCD3) in a human B-cell lymphoma xenograft.. Fichtner I (Reprint); Bargou R; Schlereth B; **Baeuerle P**; Dreier T. Expt Pharmacol & Oncol GmbH, Berlin, Germany; Robert Roessle Canc Ctr, Berlin, Germany; Micromet AG, Munich, Germany. CLINICAL CANCER RESEARCH (1 DEC 2003) Vol. 9, No. 16, Part 2, Supp. [S], pp. 6226S-6227S. ISSN: 1078-0432. Publisher: AMER ASSOC CANCER RESEARCH, 615 CHESTNUT ST, 17TH FLOOR,

PHILADELPHIA, PA 19106-4404 USA. Language: English.

L12 ANSWER 13 OF 15 MEDLINE on STN DUPLICATE 8
2003193312. PubMed ID: 12682277. T cell costimulus-independent and very efficacious inhibition of tumor growth in mice bearing subcutaneous or leukemic human B cell lymphoma xenografts by a CD19-/CD3-**bispecific single-chain antibody** construct. Dreier Torsten; **Baeuerle Patrick A**; Fichtner Iduna; Grun Michael; Schlereth Bernd; Lorenczewski Grit; Kufer Peter; Lutterbuse Ralf; Riethmuller Gert; Gjorstrup Per; Bargou Ralf C. (Micromet AG, Munich, Germany.) Journal of immunology (Baltimore, Md. : 1950), (2003 Apr 15) Vol. 170, No. 8, pp. 4397-402. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB We have recently demonstrated that a recombinant single-chain bispecific Ab construct, bscCD19xCD3, in vitro induces rapid B lymphoma-directed cytotoxicity at picomolar concentrations with unstimulated peripheral T cells. In this study, we show that treatment of nonobese diabetic SCID mice with submicrogram doses of bscCD19xCD3 could prevent growth of s.c. human B lymphoma xenografts and essentially cured animals when given at an early tumor stage. The effect was dose dependent, dependent on E:T ratio and the time between tumor inoculation and administration of bscCD19xCD3. No therapeutic effect was seen in the presence of human lymphocytes alone, a vehicle control, or with a bispecific single-chain construct of identical T cell-binding activity but different target specificity. In a leukemic nonobese diabetic SCID mouse model, treatment with bscCD19xCD3 prolonged survival of mice in a dose-dependent fashion. The human lymphocytes used as effector cells in both animal models did not express detectable T cell activation markers at the time of coinoculation with tumor cells. The bispecific Ab therefore showed an in vivo activity comparable to that observed in cell culture with respect to high potency and T cell costimulus independence. These properties make bscCD19xCD3 superior to previously investigated CD19 bispecific Ab-based therapies.

L12 ANSWER 14 OF 15 MEDLINE on STN DUPLICATE 9
2003228766. PubMed ID: 12750704. Efficient elimination of chronic lymphocytic leukaemia B cells by autologous T cells with a bispecific anti-CD19/anti-CD3 single-chain antibody construct. Loffler A; Gruen M; Wuchter C; Schriever F; Kufer P; Dreier T; Hanakam F; **Baeuerle P A** ; Bommert K; Karawajew L; Dorken B; Bargou R C. (Department of Haematology, Oncology and Tumourimmunology, Robert Rossle Clinic, Charite, Humboldt University of Berlin, Berlin, Germany.) Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K, (2003 May) Vol. 17, No. 5, pp. 900-9. Journal code: 8704895. ISSN: 0887-6924. Pub. country: England: United Kingdom. Language: English.

AB Recently, we have shown that a novel recombinant **bispecific single-chain antibody** construct (bscCD19 x CD3), induces highly efficacious lymphoma-directed cytotoxicity mediated by unstimulated peripheral T lymphocytes. Functional analysis of bscCD19 x CD3 has so far been exclusively performed with human B lymphoma cell lines and T cells from healthy donors. Here we analysed the properties of bscCD19 x CD3 using primary B cells and autologous T cells from healthy volunteers or patients with B-cell chronic lymphocytic leukaemia (B-CLL). We show that bscCD19 x CD3 induces T-cell-mediated depletion of nonmalignant B cells in all four cases and depletion of primary lymphoma cells in 22 out of 25 cases. This effect could be observed at low effector-to-target (E:T) ratios and in the majority of cases without additional activation of autologous T cells by IL-2. Even in samples derived from patients heavily pretreated with different chemotherapy regimens, strong cytotoxic effects of bscCD19 x CD3 could be observed. The addition of bscCD19 x CD3 to patients' cells resulted in an upregulation of activation-specific cell surface antigens on autologous T cells and elevated levels of CD95 on lymphoma B cells. Although anti-CD95 antibody CH-11 failed to induce apoptosis in lymphoma cells, we provide evidence that B-CLL cell depletion by bscCD3 x CD3 is mediated at least in part by apoptosis via the caspase pathway.

L12 ANSWER 15 OF 15 MEDLINE on STN DUPLICATE 10

2003157143. PubMed ID: 12673686. Efficient tumor cell lysis by autologous, tumor-resident T lymphocytes in primary ovarian cancer samples by an EP-CAM-/CD3-bispecific antibody. Wimberger Pauline; Xiang Wei; Mayr Doris; Diebold Joachim; Dreier Torsten; **Baeuerle Patrick A**; Kimmig Rainer. (Department of Gynecology and Obstetrics, University of Essen, Essen, Germany.. pauline.wimberger@med.uni-essen.de) . International journal of cancer. Journal international du cancer, (2003 Jun 10) Vol. 105, No. 2, pp. 241-8. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB The epithelial cell adhesion molecule (Ep-CAM) is expressed on the surface of most human carcinomas, including ovarian, breast, lung, prostate and colorectal carcinoma. Ep-CAM was shown to be a valid target for monoclonal antibody-based therapies. We have investigated whether an Ep-CAM-/CD3-**bispecific single-chain antibody** called bscEp-CAM x CD3 is effective in tumor cell elimination within the cellular microenvironment of primary ovarian cancer tissue. The ex vivo elimination of ovarian cancer cells in tumor preparations from 21 patients was monitored by flow cytometry using Ep-CAM/CA-125 double-labeling or Ep-CAM single-labeling combined with propidium iodide uptake of cells. Methodology was established by the ovarian cancer cell line OvCAR. A total of 17 (81%) patient samples showed a dose-dependent tumor cell elimination by bscEp-CAM x CD3. High and specific tumor cell lysis was seen at bscEp-CAM x CD3 concentrations as low as 1 ng/ml, at very low effector:target ratios and in the absence of T cell costimulation. The high efficacy of the bispecific antibody may be due to the non-restricted activation of tumor-resident cytotoxic T lymphocytes. In clinical trials, the ex vivo data with the T cell-recruiting bispecific antibody bscEp-CAM x CD3 may translate into a high response rate and efficacy of tumor cell elimination.

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L3 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2006 ACS on STN
2005:546858 Document No. 143:76820 **Bispecific single-chain antibodies** preparation and therapeutic uses thereof. Kufer, Peter; Berry, Meera; Baeuerle, Patrick; **Itin, Christian** (Germany). U.S. Pat. Appl. Publ. US 2005136050 A1 20050623, 12 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-743697 20031222.

AB The present invention discloses bispecific antibodies comprising two antibody variable domains on a single polypeptide chain, wherein a first portion of the bispecific antibody is capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen on the human immune effector cell, the first portion consisting of one antibody variable domain, and a second portion of the bispecific antibody specifically binding to a target antigen other than the effector antigen, the target antigen on a target cell other than the human immune effector cell, the second portion comprising one antibody variable domain (or 2 antibody variable domains). The inventors describe preparation and purification of a bispecific antibody with 3 antibody variable domains located on the same polypeptide chain. Progressing from the N to C terminus, the antibody contains: anti-human EpCAM VL; 15 amino acid linker of sequence (Gly4Ser)3; anti-human EpCAM VH; 5 amino acid spacer Gly4Ser; anti-human CD3 VH; His6. This antibody showed activity as a recruiter of cytotoxic T cells as shown by the efficient killing of the target cells in a manner depending on the concentrate of the bispecific antibody added to a reaction mixture in the presence of cytotoxic T cells.

L3 ANSWER 2 OF 2 MEDLINE on STN DUPLICATE 1
2005160513. PubMed ID: 15688411. Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-**bispecific single-chain antibody** construct. Hoffmann Patrick; Hofmeister Robert; Brischwein Klaus; Brandl Christian; Crommer Sandrine; Bargou Ralf; **Itin Christian**; Prang Nadja; Baeuerle Patrick A. (Micromet AG, Munich, Germany.) International journal of cancer. Journal international du cancer, (2005 May 20) Vol. 115, No. 1, pp. 98-104. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Certain bispecific antibodies exhibit an extraordinary potency and efficacy for target cell lysis by eliciting a polyclonal T-cell response.

One example is a CD19-/CD3-**bispecific single-chain antibody** construct (bscCD19xCD3), which at femtomolar concentrations can redirect cytotoxic T cells to eliminate human B lymphocytes, B lymphoma cell lines and patient-derived malignant B cells. Here we have further explored the basis for this high potency. Using video-assisted microscopy, bscCD19xCD3 was found to alter the motility and activity of T cells from a scanning to a killing mode. Individual T cells could eliminate multiple target cells within a 9 hr time period, resulting in nuclear fragmentation and membrane blebbing of target cells. Complete target cell elimination was observed within 24 hr at effector-to-target cell ratios as low as 1:5. Under optimal conditions, cell killing started within minutes after addition of bscCD19xCD3, suggesting that the rate of serial killing was mostly determined by T-cell movement and target cell scanning and lysis. At all times, T cells remained highly motile, and no clusters of T and target cells were induced by the bispecific antibody. Bystanding target-negative cells were not detectably affected. Repeated target cell lysis by bscCD19xCD3-activated T cells increased the proportion of CD19/CD3 double-positive T cells, which was most likely a consequence of transfer of CD19 from B to T cells during cytolytic synapse formation. To our knowledge, this is the first study showing that a bispecific antibody can sustain multiple rounds of target cell lysis by T cells.


```
=> s l1 and bispecific single chain
L4            7 L1 AND BISPECIFIC SINGLE CHAIN

=> dup reomve l4
ENTER REMOVE, IDENTIFY, ONLY, OR (?):remove
'REOMVE' IS NOT VALID.  VALID FILE NAMES ARE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH,
CAPLUS'
You have entered a file name of duplicates to keep that is not
referenced by any of the L#s specified for this DUPLICATE command.
The file names of duplicates that can be kept are listed above.
Please enter one of these file names.

=> remove l4
DELETE L4? (Y)/N:n

=> dup remove l4
PROCESSING COMPLETED FOR L4
L5             3 DUP REMOVE L4 (4 DUPLICATES REMOVED)

=> d l5 1-3 cbib abs

L5   ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2006 ACS on STN
2005:493633   Document No. 143:42686  Compositions comprising polypeptides
     specifically binding predetd. antigens and uses thereof.  Hofmeister,
     Robert; Prang, Nadja; Wolf, Andreas; Hanakam, Frank; Urbig, Thomas;
     Itin, Christian; Baeuerle, Patrick (Micromet A.-G., Germany).  PCT
     Int. Appl. WO 2005052004 A2 20050609, 49 pp.  DESIGNATED STATES: W:  AE,
     AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR,
     CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU,
     ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
     MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU,
     SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,
     VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK,
     ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,
     TD, TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO 2004-EP13445
     20041126.  PRIORITY: EP 2003-27511 20031128.
AB   The present invention relates to compns. comprising polypeptides capable
     of specifically binding predetd. antigens; the polypeptide in the composition
     comprises at least two antigen-binding sites.  These at least two antigen
     binding sites are located on a single polypeptide chain.  One of the at
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least two antigen binding sites specifically binds the human CD3 antigen. The polypeptide may exist in both monomeric form and multimeric form (usually dimeric). The multimeric form of the polypeptide constitutes no more than 5% of the total weight of the combined monomeric and multimeric forms of said polypeptide. One example presents activation of T cells by polypeptide in dimeric form in the absence of target cells. The results demonstrated that the dimeric polypeptide was able to activate T cells, whereas the monomer was not. These polypeptide compns. can be used for the prevention, treatment, or amelioration of diseases and disorders occurring in man.

L5 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2006 ACS on STN
2005:546858 Document No. 143:76820 **Bispecific single-chain** antibodies preparation and therapeutic uses thereof. Kufer, Peter; Berry, Meera; Baeuerle, Patrick; **Itin, Christian** (Germany). U.S. Pat. Appl. Publ. US 2005136050 A1 20050623, 12 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-743697 20031222.

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L5 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
2005160513. PubMed ID: 15688411. Serial killing of tumor cells by cytotoxic T cells redirected with a CD19-/CD3-**bispecific single-chain** antibody construct. Hoffmann Patrick; Hofmeister Robert; Brischwein Klaus; Brandl Christian; Crommer Sandrine; Bargou Ralf; **Itin Christian**; Prang Nadja; Baeuerle Patrick A. (Micromet AG, Munich, Germany.) International journal of cancer. Journal international du cancer, (2005 May 20) Vol. 115, No. 1, pp. 98-104. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

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proportion of CD19/CD3 double-positive T cells, which was most likely a consequence of transfer of CD19 from B to T cells during cytolytic synapse formation. To our knowledge, this is the first study showing that a bispecific antibody can sustain multiple rounds of target cell lysis by T cells.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	29.87	30.08

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-2.25	-2.25

STN INTERNATIONAL LOGOFF AT 14:24:00 ON 24 MAR 2006